Optimizing bacterial resource allocation: metabolite production in continuous bioreactors

Agustín Gabriel Yabo, Jean-Luc Gouzé

Optimizing bacterial resource allocation: metabolite production in continuous bioreactors ⋆

Agustín Gabriel Yabo * **Jean-Luc Gouzé** *

* *Université Côte d'Azur, Inria, INRAE, CNRS, Sorbonne Université, Biocore Team, Sophia Antipolis, France. agustin.yabo@inria.fr, jean-luc.gouze@inria.fr*

Abstract: We show preliminary results addressing the problem of resource allocation in bacteria in the continuous bioreactor framework. We propose a coarse-grained self-replicator dynamical model that accounts for the microbial population growth inside a continuous bioreactor, and we study its asymptotic behavior. This is done through a dynamical systems analysis approach, in order to provide conditions for the persistence of the bacterial population. We then study the two most relevant cases of steady-state production in this scheme: 1) biomass production, classical in high-tech industrial processes as well as in research environments; and 2) metabolite production through the introduction of a heterologous metabolic pathway. Both problems are explored in terms of the internal allocation control—which can be externally disrupted—and the constant volumetric flow of the bioreactor; and analyzed through a numerical approach. The resulting two-dimensional optimization problem is defined in terms of Michaelis-Menten kinetics using the parameter values of previous works, and taking into account the constraints for the existence of the equilibrium of interest.

Keywords: dynamics and control, modeling and identification, industrial biotechnology

1. INTRODUCTION

Microorganisms continuously face environmental changes in nature, and thus they have evolved to rapidly adapt their physiology to cope with this unsteadiness. This is achieved through reorganization of the gene expression machinery, by dynamically allocating resources to different cellular functions. Such natural allocation mechanisms have been recently addressed in Giordano et al. (2016), where the authors explored, among other things, how bacterial populations respond to changes on the nutrient concentration of the medium. This was done through the so-called coarse-grained self-replicator models, widely used in bacterial growth representations for their simplicity and their capacity to reproduce observed experimental behaviors, as shown in Koch (1988). These studies have triggered interesting questions from the biotechnological point of view, such as how to re-engineer the naturally-evolved behaviors of the cell in order to attain specific productivity objectives. Such is the subject matter addressed in Yegorov et al. (2018), Cinquemani et al. (2019) and Yabo et al. (2019), where they considered the problem of optimally producing a certain metabolite or protein of interest in a engineered strain of *Escherichia coli*. The latter is done by introducing the bacterial growth switch designed in Izard et al. (2015), that allows to externally modify the natural process of resource allocation so as to channel resources into the production of this compound of interest. As exhibited in Huo et al. (2019), the importance of such compounds arises from the potential of efficiently and sustainably producing antibiotics, antitumor agents, insecticides and immunosuppressive agents, among others. In bacteria, the production of these metabolites draws resources from the native pathways of the host cell used for synthesizing biomass, and therefore there is always a compromise between these two objectives. One approach is to model this trade-off through different cost functions, thus obtaining multi-objective optimization problems. This is done in Otero-Muras et al. (2019), where the authors seek to maximize the production of a metabolite of interest while minimizing the genetic burden caused by pathway expression. In the work of Yegorov et al. (2018), the main trade-offs behind the process are encompassed within a single decision parameter, which considerably reduces the complexity of the optimization problem. Based on these results, we address in this work a different production scheme: the Continuous Stirred-Tank Reactor (CSTR). While resource allocation in bacteria has been extensively studied in constant environments, both in steady-state and in dynamic conditions (Molenaar et al. (2009)), how this goes in continuous reactors is not immediate, as a feedback occurs from the physiology of the cell to the environmental conditions (Wortel et al. (2016)).

In this work, we show preliminary results addressing the problem of resource allocation in bacteria in the CSTR framework. We propose a coarse-grained self-replicator

⋆ This work was partially supported by ANR project Maximic (ANR-17-CE40-0024-01), Inria IPL Cosy and Labex SIGNALIFE (ANR-11-LABX-0028-01). We also acknowledge the support of the FMJH Program PGMO and the support to this program from EDF-THALES-ORANGE.

dynamical model that accounts for the microbial population growth inside a continuous bioreactor, and we study its asymptotic behavior through a dynamical systems analysis approach, in order to provide conditions for the persistence of the bacterial population. Then, we study the two most relevant cases of steady-state production in CSTRs: 1) biomass production, classical in high-tech industrial processes as well as in research environments; and 2) metabolite production, through the introduction of a heterologous metabolic pathway. Both problems are explored in terms of the internal allocation control—which can be externally disrupted—and the constant volumetric flow of the bioreactor; and analyzed through a numerical approach. The resulting two-dimensional optimization problem is defined in terms of Michaelis-Menten kinetics with the parameter values of Giordano et al. (2016), and taking into account the constraints for the existence of the equilibrium of interest.

2. MODEL DEFINITION

As previously stated, we formulate the problem of resource allocation in bacteria through coarse-grained self-replicator models. We consider a growing bacterial population in a CSTR of volume $\mathcal{V}_{ext}$. The bacterial population is represented by a self-replicating system composed of the gene expression machinery R (RNA polymerase, ribosomes...) and the metabolic machinery M (transporters, enzymes...), both responsible of the cell growth. This simplified scheme is based on the assumption that the individual cells of the growing culture share the same macromolecular composition. Additionally, we consider the extension introduced in Yegorov et al. (2019): an heterologous, artificially engineered pathway for the production of a compound of interest X (Figure 1).

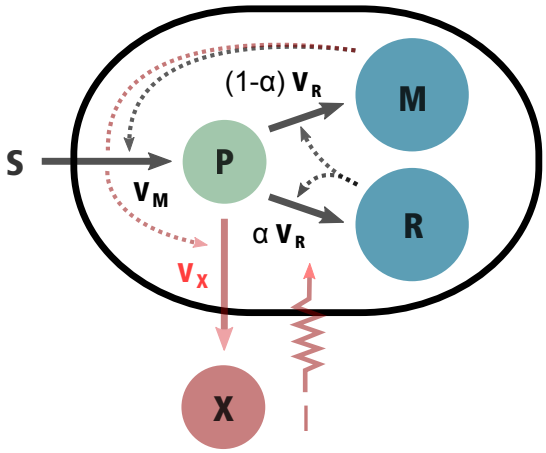


Fig. 1. Extended coarse-grained self replicator model introduced in Yegorov et al. (2019), where the external control I is able to influence how the precursors P are distributed between the metabolic machinery M and the gene expression machinery R.

The model describes essentially three chemical macroreactions

$$\mathrm{S} \xrightarrow{\mathrm{V_M}} \mathrm{P},$$
$$\mathrm{P} \xrightarrow{\mathrm{V_R}} \alpha\mathrm{R} + (1\text{-}\alpha)\,\mathrm{M},$$
$$\mathrm{P} \xrightarrow{\mathrm{V_X}} \mathrm{X}.$$

The first reaction—catalyzed by M—transforms the external substrate S into precursor metabolites P at rate V_M. Then, the precursors are converted into macromolecules R and M in a second reaction catalyzed by R, at rate V_R. The product X is also transformed from precursors with rate V_X, and is catalyzed by M. The natural resource allocation choice is modelled by the parameter $\alpha \in [0, 1]$, and represents the proportion of precursor allocated to the gene expression machinery, while $1 - \alpha$ indicates the proportion allocated to the metabolic machinery. All mass quantities S, P, M, R and X are described in grams, and the rates in grams per hour. As is classical in CSTR bioreactors, a constant volumetric flow rate F [L h^{-1}] generates both an inflow of fresh medium rich in substrate, and an outflow of biomass and produced metabolites (see Smith and Waltman (1995)). In order to externally control the cell allocation strategy, we include in our scheme the growth switch described in Izard et al. (2015). This method has been developed in a certain *E. coli* strain, by reengineering the transcriptional control of the expression of RNA polymerase, a key component of the gene expression machinery. Thus, by varying the inducer concentration in the medium, it is possible to externally disrupt the natural allocation α so as to channel resources into the production of the compound X instead of into the production of biomass. This mechanism is modeled as $u(t) = I(t)\,\alpha(t)$, $u \in [0, 1]$, where I is the external control and α the natural allocation mechanism studied in Giordano et al. (2016). While these two control functions are supposed to act independently, we are interested in obtaining the optimal combination of them. Thus, in this work, we restrict the analysis to calculate the control input u, without decoupling the individual controls. Then, the time evolution of the mass of each component can be written as

$$\begin{cases} \dot{S} = V_{S_{in}} - V_M - V_{S_{out}}, \\ \dot{P} = V_M - V_R - V_X - V_{P_{out}}, \\ \dot{M} = (1-u)\,V_R - V_{M_{out}}, \\ \dot{R} = uV_R - V_{R_{out}}, \\ \dot{X} = V_X - V_{X_{out}}, \end{cases}$$

where the inflow/outflow rates are defined as $V_{S_{out}} = DS$, $V_{P_{out}} = DP$, $V_{M_{out}} = DM$, $V_{R_{out}} = DR$, $V_{X_{out}} = DX$, $V_{S_{in}} = Fs_{in}$, being s_{in} [g L^{-1}] the nutrient concentration of the inflow of fresh medium, and D [h^{-1}] the dilution rate given by the relation $F/\mathcal{V}_{ext}$. Under the assumption that the cytoplasmic density of the cells is constant throughout the culture, we define the volume of the cell population $\mathcal{V}$ [L] as $\mathcal{V} \doteq \beta(M + R)$, where β [L g^{-1}] corresponds to the inverse of the cytoplasmic density. This definition is based on the experiments of Bremer et al. (1996) showing that macromolecules explain most of the biomass in microbial cells. Then, for the sake of convenience, quantities of the system are expressed as concentrations, $p \doteq P/\mathcal{V}$, $r \doteq R/\mathcal{V}$, $m \doteq M/\mathcal{V}$, $s \doteq S/\mathcal{V}_{ext}$, $x \doteq X/\mathcal{V}_{ext}$, where p, r and m [g L^{-1}] are intracellular concentrations—taken with respect to the cell population volume—of precursor metabolites, ribosomes (and other components of

the gene expression machinery) and metabolic enzymes respectively; and s and x [g L^{-1}] the extracellular concentrations of substrate and metabolite with respect to the volume of the bioreactor $\mathcal{V}_{ext}$ [L]. As a result, the sum of concentrations $m + r$ is equal to the constant $1/\beta$. We then define the growth rate of the bacterial population μ [h^{-1}] as the relative variation of cell volume $\dot{\mathcal{V}}/\mathcal{V}$ without considering the effect of the volumetric flow rate (i.e. setting $F = 0$). Replacing with concentrations leads to the system

$$S : \begin{cases} \dot{s} = D(s_{in} - s) - v_M(s,m)\dfrac{\mathcal{V}}{\mathcal{V}_{ext}}, \\ \dot{p} = v_M(s,m) - v_R(p,r) - v_X(p,m) - \mu(p,r)p, \\ \dot{r} = u\, v_R(p,r) - \mu(p,r)r, \\ \dot{m} = (1-u)\, v_R(p,r) - \mu(p,r)m, \\ \dot{x} = v_X(p,m)\dfrac{\mathcal{V}}{\mathcal{V}_{ext}} - Dx, \\ \dot{\mathcal{V}} = (\mu(p,r) - D)\, \mathcal{V}, \end{cases}$$

where $v_M(s,m)$, $v_R(p,r)$ and $v_X(p,m)$ [g L^{-1} h^{-1}] are the mass fluxes per unit volume obtained from dividing the rates V_M, V_R and V_X by $\mathcal{V}$, and are now function of the concentrations of system S. The growth rate becomes

$$\mu(p,r) \doteq \left.\frac{\dot{\mathcal{V}}}{\mathcal{V}}\right|_{F=0} = \left.\frac{\dot{M} + \dot{R}}{M + R}\right|_{F=0} = \beta v_R(p,r).$$

As is classical in biology, we will make some assumptions on the synthesis rates $v_M(s,m)$, $v_R(p,r)$ and $v_X(p,m)$ that describe the macroreactions:

Assumption 2.1. Functions $v_M(s,m)$, $v_R(p,r)$ and $v_X(p,m)$ meet $v_i(y,z) : \mathbb{R}^2 \to \mathbb{R}^+$, continuously differentiable w.r.t. both variables, $v_i(0,z) = v_i(y,0) = 0$, $v_i(\cdot)$ strictly monotonically increasing w.r.t. both variables: $\frac{\partial}{\partial y} v_i(y,z) > 0, \forall (y,z) \in \mathbb{R}^2_{>0}$, $\frac{\partial}{\partial z} v_i(y,z) > 0, \forall (y,z) \in \mathbb{R}^2_{>0}$, $v_i(\cdot)$ bounded w.r.t. y: $\lim_{y\to\infty} v_i(y,z) = v_{i,max}(z)$.

Assumption 2.1 encompasses all monotone increasing kinetics models, such as Michaelis-Menten. To simplify the system, we propose the following change of variables: $\hat{s} = \beta s$, $\hat{p} = \beta p$, $\hat{r} = \beta r$, $\hat{m} = \beta m$, $\hat{x} = \beta x$ and $\hat{\mathcal{V}} = \mathcal{V}/\mathcal{V}_{ext}$. The same is done for the synthesis rates $\hat{v}_M = \beta v_M$, $\hat{v}_R = \beta v_R$ and $\hat{v}_X = \beta v_X$ and for the parameter $\hat{s}_{in} = \beta s_{in}$. Then, dropping all hats yields system

$$S_1 : \begin{cases} \dot{s} = D(s_{in} - s) - v_M(s, 1-r)\mathcal{V}, \\ \dot{p} = v_M(s, 1-r) - v_X(p, 1-r) - \mu(p,r)(p+1), \\ \dot{r} = (u - r)\, \mu(p,r), \\ \dot{x} = v_X(p, 1-r)\mathcal{V} - Dx, \\ \dot{\mathcal{V}} = (\mu(p,r) - D)\, \mathcal{V}, \end{cases}$$

where the dynamical expression of m has been omitted since, as already shown, it can be expressed as $m = 1 - r$. In this first work, we will focus on a particular kind of systems where the synthesis rate related to the metabolite production depends on the growth rate:

Assumption 2.2. For $r \in (0,1)$, the metabolite production rate $v_X(p, 1-r)$ can be expressed in terms of macromolecule synthesis rate $v_R(p,r)$ as

$$v_X(p, 1-r) = c(r) v_R(p,r),$$

being $c(r) : (0,1) \to \mathbb{R}^+$ a positive continuously differentiable function.

As previously described, v_X is catalyzed by m, while v_R ($= \mu$) is catalyzed by r, which means that the balance between M and R in the cell population will determine whether the resources are being allocated to biomass growth or to metabolite production. This trade-off is modeled through the function $c(r)$, and the fact that it does not depend on p represents the assumption that the cell has the same affinity to produce both biomass and metabolite from the precursors P—even if the reactions consume the precursors in different proportions. In the particular case of Michaelis-Menten kinetics, such affinity is usually represented in the half-saturation constant, as pointed out in Johnson and Goody (2011). Indeed, one can see that, for fixed values of r, both functions are simply proportional for every value of p. Using Assumption 2.2, S_1 becomes

$$S_1 : \begin{cases} \dot{s} = D(s_{in} - s) - v_M(s, 1-r)\mathcal{V}, \\ \dot{p} = v_M(s, 1-r) - \mu(p,r)(p + c(r) + 1), \\ \dot{r} = (u - r)\, \mu(p,r), \\ \dot{x} = c(r)\mu(p,r)\mathcal{V} - Dx, \\ \dot{\mathcal{V}} = (\mu(p,r) - D)\, \mathcal{V}. \end{cases}$$

3. ASYMPTOTIC BEHAVIOR

In order to study the steady-state behavior of the system, we fix $u(t) = \bar{u} \in (0,1)$ constant. We will start the analysis of system S_1 by defining its region of operation.

Lemma 3.1. The set, $\Gamma = \{(s,p,r,x,\mathcal{V}) \in \mathbb{R}^5 : s_{in} \geq s > 0,\ p > 0,\ x \geq 0, 1 \geq r \geq 0,\ \mathcal{V} \geq 0\}$ is positively invariant for the initial value problem.

The lemma can be verified by analyzing the boundaries of Γ, which is here omitted for the sake of brevity. Moreover, we will suppose the following initial conditions for the initial value problem:

$$\begin{aligned} s_{in} \geq s(0) \geq 0,\ p(0) \geq 0,\ r(0) > 0, \\ m(0) = 1 - r(0) \geq 0,\ x(0) \geq 0,\ \mathcal{V}(0) > 0. \end{aligned} \tag{1}$$

3.2 Mass conservation

System S_1 can be rewritten as

$$\begin{cases} \dot{\varphi} = N\boldsymbol{v_i} - \boldsymbol{v_\mu}\, \mu(p,r) + D\,(s_{in}\boldsymbol{v_{in}} - \boldsymbol{v_{out}}), \\ \dot{\mathcal{V}} = (\mu(p,r) - D)\mathcal{V}, \end{cases}$$

where $\varphi \doteq [s,p,r,m,x]^T$ is the state vector of concentrations in the system, N the stoichiometry matrix of the internal reactions, v_i the vector of internal fluxes, v_{in} and v_{out} the vectors of inflows and outflows of the system respectively—associated to the external variables—and v_μ the vector of dilution due to variation of the bacterial volume—associated to the internal variables—defined as

$$N \doteq \begin{bmatrix} -\mathcal{V} & 0 & 0 \\ 1 & -1 & -1 \\ 0 & u & 0 \\ 0 & 1-u & 0 \\ 0 & 0 & \mathcal{V} \end{bmatrix}, \qquad \boldsymbol{v_i} \doteq \begin{bmatrix} v_M(s,1-r) \\ v_R(p,r) \\ v_X(p,1-r) \end{bmatrix},$$

$$\boldsymbol{v_\mu} \doteq \operatorname{diag}(\varphi)\,[0,1,1,1,0]^T,$$

$$\boldsymbol{v_{in}} \doteq [1,0,0,0,0]^T, \qquad \boldsymbol{v_{out}} \doteq \operatorname{diag}(\varphi)\,[1,0,0,0,1]^T.$$

By studying the left null space of N, it can be seen that there are two mass conservation laws related to the total mass inside the bioreactor.

Definition 3.3. We define the quantities

$$w_1 \doteq s + (p+m+r)\,\mathcal{V} + x = s + (p+1)\,\mathcal{V} + x,$$
$$w_2 \doteq s + \left(p + \frac{r}{\bar{u}}\right)\mathcal{V} + x.$$

These quantities tend asymptotically to $w_i = s_{in}$ as $t \to \infty$, since they obey the dynamical equations $\dot{w}_i = D\,(s_{in} - w_i)$ for $i = 1, 2$ which simplifies the analysis of the asymptotic behavior of the system.

Lemma 3.4. The ω-limit set of any solution of system S_1 lies in the hyperplanes

$$\Omega_1 \doteq \left\{(s,p,r,x,\mathcal{V}) \in \mathbb{R}^5 \,:\, s + (p+1)\,\mathcal{V} + x = s_{in}\right\},$$
$$\Omega_2 \doteq \left\{(s,p,r,x,\mathcal{V}) \in \mathbb{R}^5 \,:\, s + \left(p + \frac{r}{\bar{u}}\right)\mathcal{V} + x = s_{in}\right\}.$$

Further on, we will use Lemma 3.4 to analyze system S_1 through its limiting system.

3.5 Limiting systems

Lemma 3.4 involves two mass conservation laws, so it can be used to reduce subsystem S_1 by two dimensions. We will start by analyzing the asymptotic behavior of r: when $t \to \infty$, $w_1 = w_2$, and then

$$s + (p+1)\,\mathcal{V} + x = s + \left(p + \frac{r}{\bar{u}}\right)\mathcal{V} + x \quad \Rightarrow \quad r = \bar{u}$$

meaning that, as $t \to \infty$, r will converge to the value $\bar{u}$. Additionally, we can express $x = s_{in} - s - (p+1)\mathcal{V}$ and so the limiting system of S_1 becomes

$$S_1' \,:\, \begin{cases} \dot{s} = D(s_{in} - s) - \bar{v}_M(s)\mathcal{V}, \\ \dot{p} = \bar{v}_M(s) - \bar{\mu}(p)(p + \bar{c} + 1), \\ \dot{\mathcal{V}} = (\bar{\mu}(p) - D)\,\mathcal{V}, \end{cases}$$

with flows $\bar{v}_M(s) \doteq v_M(s, 1-\bar{u})$, $\bar{v}_R(p) \doteq v_R(p, \bar{u})$, $\bar{v}_X(p) \doteq v_X(p, 1-\bar{u})$ and $\bar{c} \doteq c(\bar{u})$. Convergence of the limiting system S_1' to the original S_1 will be addressed later, after fully describing the asymptotic behavior of S_1'.

3.6 Local stability

For notation purposes, we will start the analysis defining the following function.

Definition 3.7. We define the function

$$\bar{f}(p) \doteq \bar{v}_R(p) + \bar{v}_X(p) + \bar{\mu}(p)p = \bar{\mu}(p)(p + \bar{c} + 1).$$

We note that $\bar{f}(p) > 0, \bar{f}'(p) > 0, \forall p \in \Gamma$ (positive and monotonically increasing). Then, the local stability of the system is given by the following lemma.

Proposition 3.8. System S_1' can admit two equilibria: the interior equilibrium $E_i \doteq (s_i, p_i, \mathcal{V}_i)$, and the washout equilibrium $E_w \doteq (s_{in}, p_w, 0)$ where

$$p_i : \{p \in \mathbb{R} \,:\, \bar{\mu}(p) = D\}, \tag{2}$$
$$p_w : \left\{p \in \mathbb{R} \,:\, \bar{f}(p) = \bar{v}_M(s_{in})\right\}, \tag{3}$$
$$s_i : \left\{s \in \mathbb{R} \,:\, \bar{v}_M(s) = \bar{f}(p_i)\right\}, \tag{4}$$
$$\mathcal{V}_i = \frac{D(s_{in} - s_i)}{\bar{v}_M(s_i)}. \tag{5}$$

Moreover, if values p_i, p_w and s_i exist, they are unique.

Proof. The uniqueness of p_i in $\bar{\mu}(p) = D$ comes from the fact that $\bar{\mu}(p)$ is strictly monotonically increasing w.r.t. p and so, if there exist an intersection between $\bar{\mu}(p)$ and D, it should be unique. A similar argument can be used in $\bar{v}_M(s) = \bar{f}(p_i)$ and $\bar{f}(p) = \bar{v}_M(s_{in})$, where both functions are also strictly monotonically increasing so, if there exist an intersection between the functions and the constants, it should be unique.

It is possible to find bounds on p by defining a time-varying upper bound $p_{up}(t)$ with dynamical equation $\dot{p_{up}} \leq \bar{v}_M(s_{in}) - \bar{f}(p_{up})$. In this case, p_{up} converges towards the equilibrium point $p = p_w$ that satisfies (3). Moreover, the vector field at $p = p_w$ is null, and so a new invariant set $\Gamma' \subset \Gamma$ can be defined as

$$\Gamma' = \left\{(s,p,\mathcal{V}) \in \mathbb{R}^3 \,:\, s_{in} \geq s > 0,\, p_w \geq p > 0,\, \mathcal{V} \geq 0\right\}.$$

To sum up, the local behavior of equilibria can be described as follows:

- If $\bar{\mu}(p_w) \geq D$:
  - E_i exists and is locally stable
  - E_w exists and is locally unstable
- If $\bar{\mu}(p_w) < D$:
  - E_i does not exist
  - E_w exists and is locally stable

where the details of the analysis are omitted due to lack of space.

3.9 Global analysis

Using the theory of asymptotically autonomous systems developed in Thieme (1992), it is possible to establish that the limiting system S_1' has the same asymptotic behavior as the full 5-dimensional system S_1. However, a thorough global stability analysis of the limiting system S_1' is omitted in this work. Asymptotically autonomous systems theory ensures that almost all trajectories of the original system S_1 converge to one of the asymptotically stable rest points of the limiting system. As there is always one stable equilibrium, it is straightforward to formalize the latter.

Theorem 3.10. Every solution of system S_1 with initial conditions (1) will converge to

- The interior equilibrium E_i if it exists, and the metabolite concentration to $x = x_i \doteq \bar{c}\mathcal{V}_i$.
- The washout equilibrium E_w if E_i does not exist, and the metabolite concentration to $x = 0$.

4. STATIC OPTIMIZATION PROBLEM

4.1 Biomass or product maximization

The static biomass maximization problem (BMP) can be written as

$$(BMP) : \begin{cases} \textit{maximize} & J_{\mathcal{V}}(\bar{u}, D) \doteq D\mathcal{V} \\ \textit{subject to} & (2), (4), (5), \\ \textit{and} & 0 \leq \bar{u} \leq 1. \end{cases}$$

Analogously, the product maximization problem can be defined as

$$(PMP) : \begin{cases} \textit{maximize} & J_x(\bar{u}, D) \doteq Dx \\ \textit{subject to} & (2), (4), (5), \\ \textit{and} & 0 \leq \bar{u} \leq 1. \end{cases}$$

4.2 Interior solution

As we are looking for the steady-states that maximize each objective, we can immediately rule out the washout equilibrium E_w as a potential solution since, as shown in Lemmas 3.8 and 3.10, the point corresponds to $\mathcal{V} = 0$ and $x = 0$. Therefore, the problem reduces to find the equilibrium E_i in terms of the pair $(D, \bar{u})$ that maximizes each objective. As a first step, we can state that the optimal solution cannot belong to the boundary of the equilibrium E_i.

Proposition 4.3. A solution of the static optimization problems cannot belong to the boundary set

$$\Theta \doteq \left\{(\bar{u}, D) \in \mathbb{R}^2 : \mu(p_w, \bar{u}) = D\right\},$$

which corresponds to the set of equilibria E_i with maximal growth rate.

Proof. Using (2), (4) and (5), it can be seen that on the boundary $\mu(p_w, \bar{u}) = D$ there is no bacterial population, as $s_i = s_{in}$, which means that $\mathcal{V}_i = x_i = 0$. Then, both costs $J_{\mathcal{V}}$ and J_x would vanish, which is readily not optimal.

We recall that in the case of constant environmental conditions studied in Yegorov et al. (2018), describing fed-batch cultivation, the solution for the static maximization problem, both for biomass and metabolite production, corresponded to the steady-state with maximal growth rate. As shown in Lemma 4.3, this is not the case in continuous bioreactors, as the maximal growth rate lies within the boundary set of existence of the interior equilibrium.

5. RESULTS

In order to further explore the solution of the static optimization problems, we define the rates according to Michaelis-Menten kinetics, and use the parameter values of Giordano et al. (2016). Let us first recall that, at the interior equilibrium, the growth rate $\mu(p_i, \bar{u})$ is equal to D, as stated in (2). We illustrate the results in a similar fashion both in Figures 2 and 3: the region of existence of the interior equilibrium E_i is delimited by the set Θ of maximum growth rate, and the different values of the cost functions are depicted using a qualitative colormap to highlight the regions closer to the optimal points. Moreover, the curves $\bar{u}_{opt}(D)$ show the optimal strategy for each objective for every fixed value of D. We first solve the biomass maximization objective, shown in Figure 2. The solution is characterized by an allocation strategy that, for all values of the growth rate, remains mainly geared towards the synthesis of components of the gene expression machinery R, as $\bar{u} > 0.5$ for all values of D. Moreover, the optimal point turns out to have a fairly high growth rate ($\approx 85\%$ of the maximal growth rate). For the product maximization case, shown in Figure 3, we see that, in contrast to the first case, the optimal strategy involves allocating as much resources as possible into the metabolic machinery M, independently of the growth rate, which is consistent with the fact that the metabolic machinery catalyzes the production of the metabolite X. It is interesting to note that, in opposition to the previous case, the optimal point involves a quite lower dilution rate D, thus resulting in a continuous production at a growth rate of about 35% of the maximal growth rate. This result might be found counter-intuitive, as it is well established that increasing the dilution rate in continuous reactors leads to an increase in the production. We can attribute this particularity to the trade-off between allocating resources to the metabolic machinery and increasing the dilution rate, which is linked to the maximum value of the function $c(r)$. This same effect can be further observed in Figure 4a, where we show numerical results for the synthesis rates for each solution of (BMP) and (PMP). Indeed, both precursor and biomass synthesis rates are considerably diminished in order to increase the production of the metabolite, which is to a great extent due to this reduction of the dilution rate D in the solution of (PMP). We see how this difference in the internal resource distribution has an impact on the mass quantities inside the bioreactor at steady-state—depicted in Figure 4b—for each of the problems: while there is no substantial difference in biomass $M+R$ between solutions, we can see that for the product maximization problem, the amount of metabolite in the bioreactor corresponds to 5 times that of the biomass maximization problem.

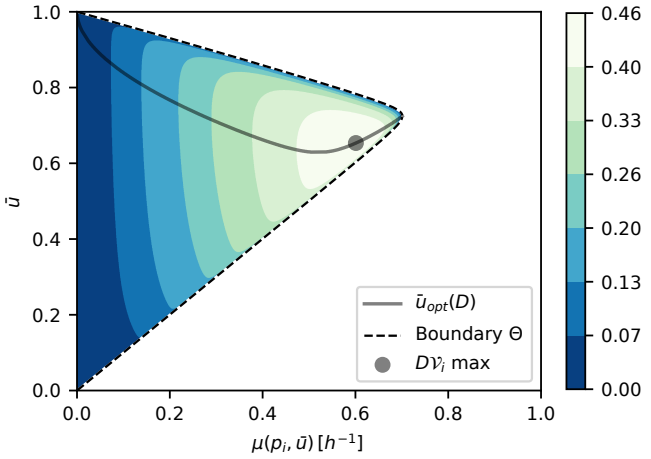


Fig. 2. Results associated to (BMP) with $J_{\mathcal{V}} = D\mathcal{V}$.

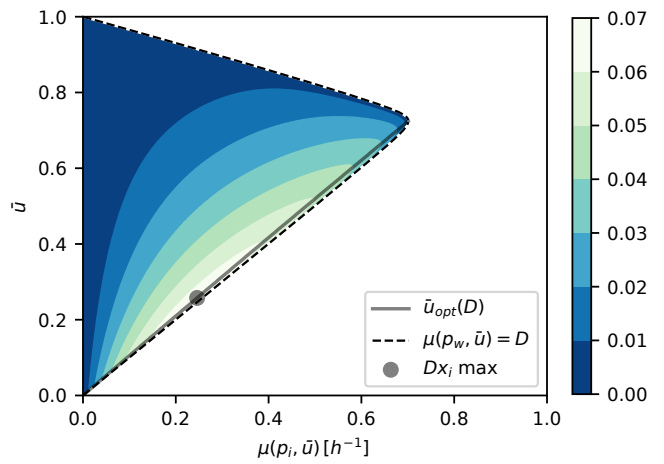
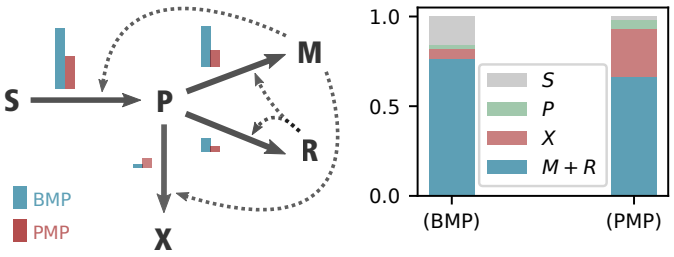


Fig. 3. Results associated to (PMP) with $J_x = Dx$.



(a) Synthesis rates magnitudes (b) Percentage mass quantities

Fig. 4. Numerical results for both static problems.

6. DISCUSSION

Our scope in this work was to synthesize a compound of interest by re-engineering the internal allocation mechanisms of certain growing population of cells. We particularly addressed this problem in the CSTR framework, where it is possible to continuously produce such metabolite in a steady-state regime. We tackled the problem through a dynamical systems approach, by using a coarse-grained whole-cell model contained inside a continuous bioreactor, for which we gave conditions for the persistence of the microbial population. Then, we compared the cases of optimally producing biomass $\mathcal{V}$, as well as a compound of interest X, through a multi-variable optimization approach in terms of the dilution rate of the bioreactor D, and the internal allocation of the cell population. While the details on how to alter the natural allocation process through the external control are out of the scope of this paper, we showed that the solutions for each problem differ widely, both in the allocation strategy as well as in the optimal microbial growth rate. These results are of great interest to biotechnological applications, as they could help enhancing productivity measures of certain bioprocesses by channeling resources to specific cellular functions. Numerous details were omitted in this first approach, such as specifics of the local stability analysis, as well as the details on the convergence of the limiting systems to the original system. The work will be eventually extended with an analysis regarding the impact of Assumption 2.2 on the results, as well as the sensitivity of the strategies to the biological parameters, both analytically and experimentally. According to our analysis, the results were robust to such issues, but at this point a thorough study is required.

ACKNOWLEDGEMENTS

We thank Jean-Baptiste Caillau (Université Côte d'Azur / Inria, McTao team) for discussions on the manuscript; and Carlos Martínez and Lucie Chambon (Inria, Biocore team) for discussions on the dynamical analysis of the system.

REFERENCES

Bremer, H., Dennis, P.P., et al. (1996). Modulation of chemical composition and other parameters of the cell by growth rate. *Escherichia coli and Salmonella: cellular and molecular biology*, 2(2), 1553–69.

Cinquemani, E., Mairet, F., Yegorov, I., de Jong, H., and Gouzé, J.L. (2019). Optimal control of bacterial growth for metabolite production: The role of timing and costs of control. In *2019 18th European Control Conference (ECC)*, 2657–2662. IEEE.

Giordano, N., Mairet, F., Gouzé, J.L., Geiselmann, J., and De Jong, H. (2016). Dynamical allocation of cellular resources as an optimal control problem: novel insights into microbial growth strategies. *PLoS computational biology*, 12(3), e1004802.

Huo, L., Hug, J.J., Fu, C., Bian, X., Zhang, Y., and Müller, R. (2019). Heterologous expression of bacterial natural product biosynthetic pathways. *Natural product reports*.

Izard, J., Balderas, C.D.G., Ropers, D., Lacour, S., Song, X., Yang, Y., Lindner, A.B., Geiselmann, J., and De Jong, H. (2015). A synthetic growth switch based on controlled expression of rna polymerase. *Molecular systems biology*, 11(11), 840.

Johnson, K.A. and Goody, R.S. (2011). The original Michaelis constant: translation of the 1913 Michaelis–Menten paper. *Biochemistry*, 50(39), 8264–8269.

Koch, A.L. (1988). Why can't a cell grow infinitely fast? *Canadian journal of microbiology*, 34(4), 421–426.

Molenaar, D., Van Berlo, R., De Ridder, D., and Teusink, B. (2009). Shifts in growth strategies reflect tradeoffs in cellular economics. *Molecular systems biology*, 5(1).

Otero-Muras, I., Mannan, A.A., Banga, J.R., and Oyarzún, D.A. (2019). Multiobjective optimization of gene circuits for metabolic engineering. *IFAC-PapersOnLine*, 52(26), 13–16.

Smith, H.L. and Waltman, P. (1995). *The theory of the chemostat: dynamics of microbial competition*, volume 13. Cambridge university press.

Thieme, H.R. (1992). Convergence results and a poincaré-bendixson trichotomy for asymptotically autonomous differential equations. *Journal of mathematical biology*, 30(7), 755–763.

Wortel, M.T., Bosdriesz, E., Teusink, B., and Bruggeman, F.J. (2016). Evolutionary pressures on microbial metabolic strategies in the chemostat. *Scientific reports*, 6, 29503.

Yabo, A., Caillau, J.B., and Gouzé, J.L. (2019). Singular regimes for the maximization of metabolite production. In *CDC 2019-58th IEEE Conference on Decision and Control*.

Yegorov, I., Mairet, F., De Jong, H., and Gouzé, J.L. (2018). Optimal control of bacterial growth for the maximization of metabolite production. *Journal of mathematical biology*, 1–48.

Yegorov, I., Mairet, F., De Jong, H., and Gouzé, J.L. (2019). Optimal control of bacterial growth for the maximization of metabolite production. *Journal of mathematical biology*, 78(4), 985–1032.